View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Open Archive Toulouse Archive Ouverte

Open Archive Toulouse Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of some Toulouse researchers and makes it freely available over the web where possible.

This is an author's version published in: https://oatao.univ-toulouse.fr/22855

Official URL :

To cite this version :

Plihon, Florence and Taillandier, Patricia and Strehaiano, Pierre *Oxygen effect on lactose catabolism by a Leuconostoc mesenteroides strain: modeling of general O2-dependent stoichiometry*. (1996) Biotechnology and Bioengineering, 49 (1). 63-69. ISSN 0006-3592

Any correspondence concerning this service should be sent to the repository administrator:

tech-oatao@listes-diff.inp-toulouse.fr

Oxygen Effect on Lactose Catabolism by a *Leuconostoc mesenteroides* Strain: Modeling of General O_2-Dependent Stoichiometry

Florence Plihon,* Patricia Taillandier, and Pierre Strehaiano
Laboratoire de Génie Chimique CNRS URA 192, ENSIGC, 18 Chemin de la Loge, 31078 Toulouse cedex, France

Lactose metabolism of a *Leuconostoc mesenteroides* strain was studied in batch cultures at a pH of 6.5 and 30°C in 10 L of a modified MRS (De Man, Rogosa, Sharp) broth. The end products of this heterolactic bacterium were D-lactate, acetate, ethanol, and carbon dioxide. To test the effect of oxygen on their synthesis, the medium was sparged with different gases: nitrogen, air, and pure oxygen. When oxygen was available, oxygen uptake occurred, which caused a modification in acetate and ethanol production but not in lactate or carbon dioxide production; acetate plus ethanol together were produced in constant amounts, which were independent of the level of aeration. The influence of oxygen on end-product formation could be summed up by the general equation: lactose + $x\ O_2 \rightarrow 2$ D-lactate + $(x + 0.1)$ acetate + $(2 - x)$ ethanol + $2\ CO_2$. Maximal oxygen uptake ($x = 2$) was reached under a 120 L/h flow rate of pure oxygen. In addition, this equation provided useful information on the possible pathway of galactose catabolism by a heterofermentative microorganism. © 1996 John Wiley & Sons, Inc.

Key words: *Leuconostoc mesenteroides* • lactose catabolism • oxygen • stoichiometry

INTRODUCTION

Glucose dissimilation under anaerobiosis by *Leuconostoc mesenteroides* species was first established by DeMoss et al.[7] and confirmed by further studies.[2,8–10] These heterofermentative microorganisms catabolize glucose via the pentose phosphoketolase pathway yielding essentially equimolar amounts of D-lactate, carbon dioxide, and ethanol under anaerobiosis.

In the presence of oxygen, *Leuconostoc mesenteroides* cultures are able to assimilate oxygen and then modify carbohydrate metabolism, according to the studies of several workers.[11,13,18] Some enzymes, such as NADH oxidases, NADH peroxidase, and acetate kinase become activated; others, such as alcohol dehydrogenase, are repressed by aerobiosis.[13] D-Lactate and carbon dioxide production are not affected by the presence of oxygen. But acetate synthesis, an energy-rich phosphate pathway, increases substantially, to the detriment of ethanol formation. Among all the investigations of the oxygen effect on the end products of *Leuconostoc* species, it appears that most authors determine the stoichiometric coefficients of the end-products for only a fixed level of aeration. A general O_2-dependent equation of sugar metabolism does not seem to have been established on *Leuconostoc* strains, or even more generally, on lactic acid bacteria.

Investigations were undertaken in this study with a *L. mesenteroides* strain to establish a general O_2-dependent equation for lactose metabolism, whichever the aerobic conditions used, and to determine the possible pathway of galactose metabolism by this microorganism. Batch cultures were operated at different oxygen transfer rates. Oxygen uptake and molar concentration of all the end products were determined during each batch culture.

MATERIALS AND METHODS

Organism and Inoculum

A strain of *L. mesenteroides* subsp. *mesenteroides*, a heterofermentative microorganism, was obtained from an industrial laboratory. The strain was stored at −18°C in MRS medium[6] (Biokar Diagnostics, Beauvais, France) with a 10% (v/v) admixture of glycerol. MRS (De Man, Rogosa, Sharp) broth (100 mL) inoculated with this admixture (4 mL) was incubated for a period of 7 h in a 250-mL Erlenmeyer flask at 30°C with magnetic agitation at 250 rpm. The fermentor was inoculated with 0.23% (v/v) of this culture.

Batch Cultures: Experimental Procedure

A 15-L fermentor (Applikon, 3100 AC Schiedam, The Netherlands) was connected to a biocontroller (ADI 1030, Applikon) from which pH and dissolved oxygen tension could be visualized. The pH of the medium was monitored with a heat-sterilizable electrode (Ingold, CH-8902 Urdorf, Switzerland). When the pH fell below the set point, the biocontroller activated a pump which provided the medium

* To whom all correspondence should be addressed.

with a 5N ammoniacal solution to neutralize the acid formed. The dissolved oxygen tension was measured with a membrane-covered polarographic oxygen electrode (Ingold) which also was heat-sterilizable. The "zero" was calibrated by sparging nitrogen, and the full scale, by sparging air, or pure oxygen when growth occurred under pure oxygen, into the sterilized medium. A water bath was used to maintain the temperature constant. The stirrer speed was controlled by an independent element (ADI 1020, Applikon). A mass flow meter (El-flow series F100/200, Bronkhorst, 7261 AK Ruurlo, The Netherlands) accurately controlled the inlet gas flow. To avoid contamination, air was introduced through a 0.2-μm Teflon filter (Sartorius, 3400 Goettingen, Germany).

Fixed Culture Parameters

For each batch culture, the temperature was fixed at 30°C, the stirrer speed at 400 rpm, and the pH at 6.5. An antifoam was added to the medium which was saturated with oxygen just before inoculation. Ten liters of modified MRS broth (20 g/L glucose was replaced by 40 g/L lactose and Tween 80 was omitted) were sterilized by heating for 55 min at 120°C. Samples were taken every hour during the lag phase and every half hour thereafter.

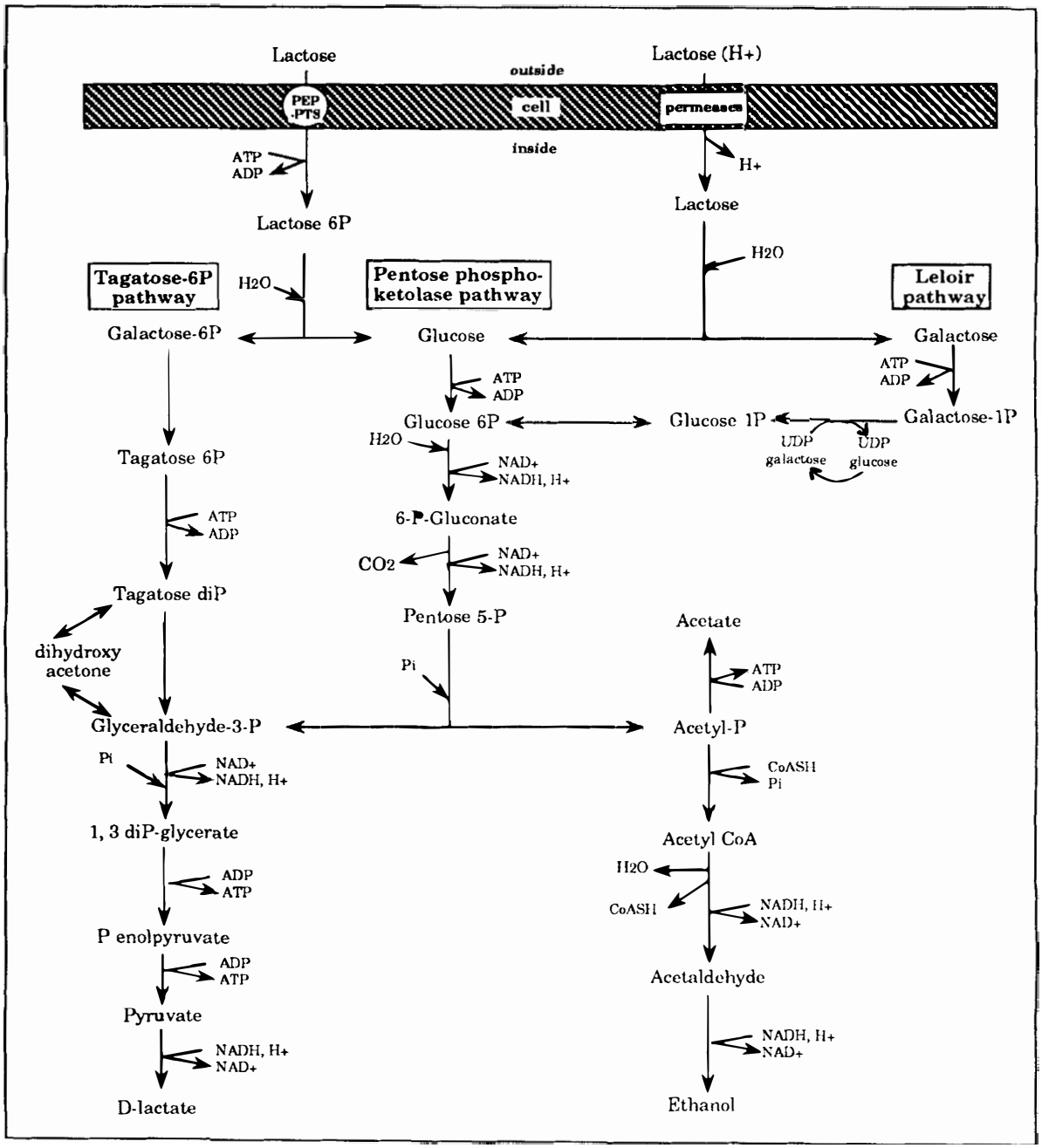

Figure 1. Possible biochemical pathways of lactose breakdown by heterolactic bacteria.

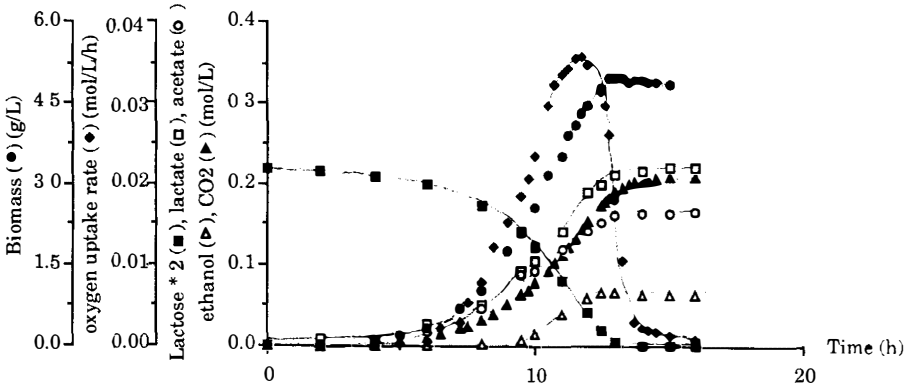

Figure 2. Growth, lactose uptake, oxygen uptake rate, and end product formation during a batch culture of *L. mesenteroides* under a 36 L/h flow rate of pure oxygen.

Variables

To test the effects of oxygen on the end products, the oxygen transfer rate OTR = kla · (C^*-C) into the medium (see Nomenclature) was progressively increased by acting on the kla and C^*. The kla was first increased by sparging the medium with different air flow rates: 60, 120, and 240 L/h, corresponding to kla values, respectively, of 26, 39, and 62/h. Pure oxygen was used to further increase the OTR: thus C^* was increased fivefold. Two flow rates were studied: 36 and 120 L/h. The condition of anaerobiosis was created by sparging the medium continuously with a 60 L/h flow rate of nitrogen.

Analysis of Outlet Gas

Oxygen uptake and carbon dioxide production were measured on-line by means of a paramagnetic gaseous oxygen analyzer (570A model, Servomex, Crowborough, Great-Britain) and an IR carbon dioxide analyzer (series 1400 model, Servomex), respectively. Two cases were examined:

- When sparging the medium with air, the general expression of the oxygen uptake rate (OUR) given by Cooney et al.[5] was simplified by: OUR = $(0.266 \times F_N - P_{O,out} \cdot F_{out})/V$ with $F_{out} = F_N/(1 - P_{O,out} - P_{C,out})$. A similar expression could be written for the carbon dioxide production rate (CPR): (CPR) = $P_{C,out} \cdot F_{out}/V$ (see Nomenclature).
- When sparging the medium with pure oxygen, these equations, based on the conservation of inert gas in the gas flow, became unavailable. Experiments showed that F_{in} F_{out}. In this case, OUR = $(1 - P_{O,out}) \cdot F_{in}/V$ and CPR = $P_{C,out} \cdot F_{in}/V$.

For each inlet gas, instantaneous oxygen uptake OU(t) and carbon dioxide production CP(t) were known, respectively, by integrating OUR(t) and CPR(t).

Analytical Methods

D-Lactate, ethanol, and carbon dioxide are the main end products of *L. mesenteroides* during lactose metabolism. A high pressure liquid chromatograph (HPLC) with a specific column (Aminex HPX-87H, Bio-Rad Laboratories, Richmond CA) maintained at 30°C separated the lactate and the acetate. Detection of each component was accomplished by means of a refractometer. The mobile phase was a 0.01N sulfuric acid solution whose rate was fixed at 0.3 mL/min. Ethanol was determined by gas chromatography (Chrompack international B.V., 4330 EA Middelburg, The Netherlands) using a column Chrompack 437A Poraplot Q wide-bore (0.53 mm × 25 m). The temperature of the heated column was 170°C; the temperature of the injector and detector was 200°C. The carrier gas was nitrogen. A 1% (v/v) isopropanol solution represented the internal standard and was added to the samples in the proportion of 2 vol of isopropanol for 1 of sample. Lactose was analyzed by means of a YSI enzymatic method and H_2O_2 was analyzed by means of a YSI electrochemical reaction (Yellow Springs Instruments, Yellow Springs, OH).

Correction on Medium Volume

The pH control induced an increase in the medium volume. Indeed, the error on the volume reached 6.6% when the volume increase was neglected for the batch cultures under pure oxygen. Besides, biomass and products concentrations did not refer to the same volume, for each fermentation, because instantaneous volume $V(t)$ varied with aeration. All experimental molar concentrations $M_i(t)$ obtained were corrected as follows: $MC_i(t) = M_i(t) \cdot V(t)/10$. Finally, each

Table I. D-Lactate and CO_2 production for each batch culture.

Aeration	D-Lactate (mol/L)	CO_2 (mol/L)
Anaerobiosis	0.219	0.217
Air		
60 L/h	0.214	0.211
120 L/h	0.224	0.219
240 L/h	0.217	0.206
O_2, 36 L/h	0.221	0.208
O_2, 120 L/h	0.226	0.209

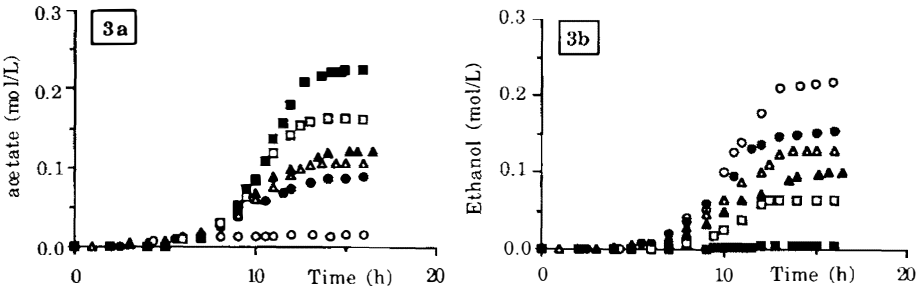

Figure 3. (a) Acetate and (b) ethanol synthesis for: (○) anaerobiosis; (●) air, 60 L/h; (△) air, 120 L/h; (▲) air, 240 L/h; (□) O_2, 36 L/h; (■) O_2, 120 L/h.

concentration had the same reference with respect to the volume (i.e., initial volume) and comparisons could then be done.

Reproducibility of Results

The batch culture under a 120 L/h air flow rate was conducted in duplicate. Growth and end products were similar for both the experiments (data not shown).

Errors on Experimental Setup and Analytical Methods

The errors were inferior to 1% for the mass flow meter, 0.1% and 1%, respectively for the O_2 and CO_2 analyzers according to the manufacturers and confirmed by experimental measures. An experimental error of 5% was generally obtained on the concentrations given by the HPLC. The experimental error was inferior to 5% for the YSI method.

THEORETICAL ASPECTS

In the metabolism of *Leuconostoc* species, the carbohydrates are completely converted into end products and so are not involved in cell elaboration.[3] Lactose is taken up from the medium to be hydrolyzed into glucose and galactose inside the cell. Although glucose breakdown by heterofermentative lactic acid bacteria is well-known[2,7–10] today, very few investigations have been undertaken to determine galactose assimilation by these microorganisms. Premi et al.[15] show that both tagatose 6-P and Leloir pathways[12] are active in the strain of *Lactobacillus brevis* XI NCDO 473 and Romano et al.[16] show that strains of *L. brevis* and *L. buchneri* lack a PEP-dependent phosphotransferase system (PTS-PEP) for galactosides transport within the cell. Possessing the two pathways seems to be a common property to lactic acid bacteria because tagatose 6-P and Leloir pathways are both active among homofermentative microorganisms.[1,15,17] Figure 1 sums up the conclusions of all these studies and provides Equation (1):

$$\text{lactose} + 2x\ \text{NAD}^+ + w\ \text{H}_2\text{O} \longrightarrow a\ \text{D-lactate} + b\ \text{acetate} + c\ \text{ethanol} + d\ \text{CO}_2 + 2x\ \text{NADH, H}^+ \quad (1)$$

When oxygen is available, NADH oxidases are alternative mechanisms of NAD^+ regeneration.[4] Two main types have been reported among *Leuconostoc* strains: NADH:H_2O oxidase and NADH:H_2O_2 oxidase which catalyze the reduction of oxygen, respectively, to H_2O (2) and H_2O_2 (3):

$$\text{NADH, H}^+ + 1/2\ \text{O}_2 \xrightarrow{\text{NADH:H}_2\text{O oxidase}} \text{NAD}^+ + \text{H}_2\text{O} \quad (2)$$

$$\text{NADH, H}^+ + \text{O}_2 \xrightarrow{\text{NADH:H}_2\text{O}_2\text{ oxidase}} \text{NAD}^+ + \text{H}_2\text{O}_2 \quad (3)$$

Independent of the type of NADH oxidases activated in

Table II. Initial and final balances on C, H, and O for each batch culture.

	Balances on C (mol/L)		Balances on H (mol/L)		Balances on O (mol/L)	
Aeration	Initial	Final	Initial	Final	Initial	Final
Anaerobiosis	1.33	1.37	2.44	2.69	1.22	1.40
Air						
60 L/h	1.34	1.39	2.46	2.61	1.40	1.48
120 L/h	1.34	1.41	2.46	2.56	1.45	1.53
240 L/h	1.32	1.32	2.42	2.41	1.32	1.45
O_2, 36 L/h	1.33	1.33	2.44	2.38	1.52	1.48
O_2, 120 L/h	1.32	1.37	2.42	2.31	1.60	1.59

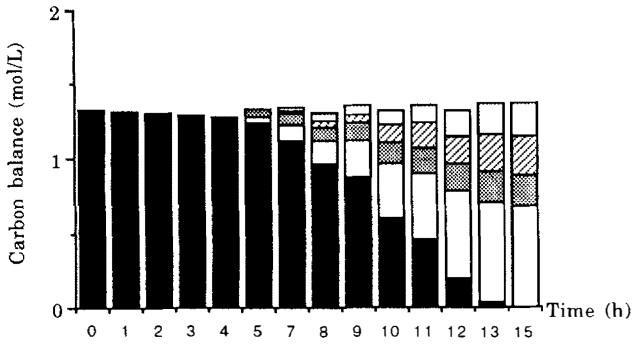

Figure 4. Carbon balance during a batch culture of *L. mesenteroides* under a 120 L/h air flow rate: (■) lactose uptake; (□) lactate, (□) acetate, (□) ethanol, and (□) carbon dioxide produced.

aerobiosis, oxygen is involved in these equations and Equation (1) coupled with Equations (2) or (3) leads to Equation (4) where oxygen is taken into account:

$$\text{lactose} + x\,O_2 \rightarrow a\ \text{D-lactate} + b\ \text{acetate} + c\ \text{ethanol} + d\,CO_2 \quad (4)$$

H_2O is omitted because its production cannot be determined by experimental data. H_2O_2 concentration was negligible (inferior to 1 m*M* independent of the level of aeration used) compared to the concentration of the other end products when reaching the stationary phase. Stoichiometric coefficients of this equation were thereafter identified via experimental data.

RESULTS AND DISCUSSION

Growth and End Products

Six batch cultures with different values of the oxygen transfer rate were conducted to determine the influence of dissolved oxygen on oxygen uptake and each end-product formation. An example of a fermentation under a 36 L/h flow rate of pure oxygen is given on Figure 2. Each level of aeration studied led to the same growth profile: a lag phase of 5 h, a quick growth phase, and a stationary phase reached at 14 h. The effect of oxygen on growth was studied in a previous article.[14] Lactate and carbon dioxide production were practically equal for a 36 L/h flow rate of pure oxygen. This property was found to be the same independent of the level of aeration studied (Table I). But acetate and ethanol production varied with the oxygen uptake (Fig. 3). Acetate synthesis increased with oxygen uptake while ethanol production decreased. Under a 120 L/h flow rate of pure oxygen, the switch was practically complete: almost no ethanol was produced. Under anaerobiosis, small amounts of acetate were produced, which confirmed the results of Lucey[13] and Yashima et al.[18]

Table II gives the balances on C, H, and O (see Nomenclature for the definition) for each batch culture and Figure 4 details the C balance for a 120 L/h air flow rate. The C balance confirmed that biomass was not created from sugar metabolism. Differences between initial and final H balances or between initial and final O balances remained low. This result showed that H_2O produced (or consumed) by reaction (4) seemed negligible compared to the synthesis of each end product.

Determination of D-Lactate and Carbon Dioxide Coefficients

Knowing the D-lactate concentration and lactose consumption for each batch culture, instantaneous variations of the D-lactate coefficient were calculated for each batch culture and summarized in Figure 5a. The same argument was applied to carbon dioxide (Fig. 5b). During the first 7 h lactose consumption remained very low (see Fig. 2): the values of each coefficient were not meaningful. After 7 h, the two coefficients kept the same constant value of 2. D-Lactate and carbon dioxide production remained independent of the oxygen uptake. These values provided useful information about the pathway used by the galactose catabolism for the strain studied. Indeed, the Leloir pathway, by joining the pentose phosphoketolase pathway with the synthesis of glucose-6P, doubles the molar production of lactate, CO_2, and acetyl-P coming from glucose catabolism (see Fig. 1). While the tagatose-6P pathway, by joining the pentose phosphoketolase pathway with the synthesis of glyceraldehyde-3P, leads to a final production of only 1 mol CO_2 and 1 mol acetyl-P/mol lactose. Of the two pathways, the Leloir pathway seems to be the most likely pathway for this strain of *L. mesenteroides*.

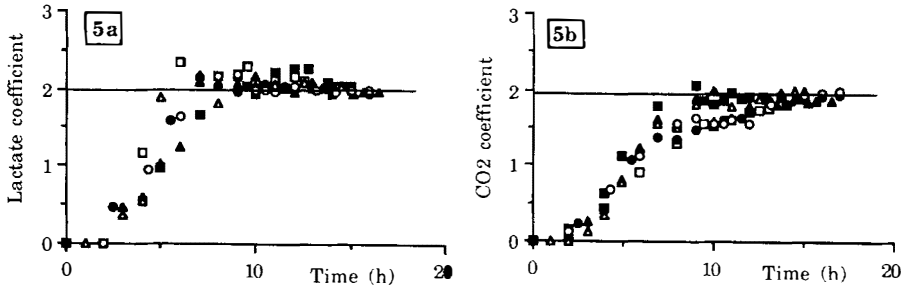

Figure 5. (a) Lactate and (b) carbon dioxide coefficients for: (○) anaerobiosis; (●) air, 60 L/h; (△) air, 120 L/h; (▲) air, 240 L/h; (□) O_2, 36 L/h; (■) O_2, 120 L/h.

Table III. Oxygen (x), acetate (b), and ethanol (c) coefficients for each batch culture.

Aeration	x	b	c
Anaerobiosis	0	0.15	1.95
Air			
60 L/h	0.63	0.79	1.39
120 L/h	0.90	0.96	1.16
240 L/h	1.06	1.10	0.91
O_2, 36 L/h	1.32	1.47	0.58
O_2, 120 L/h	1.72	2.00	0.05

Variations of Acetate and Ethanol Coefficients with Oxygen Uptake

Contrary to D-lactate and CO_2 coefficients, acetate (b) and ethanol (c) coefficients varied with oxygen uptake. Table III shows a proportional relationship between oxygen, acetate, and ethanol coefficients, modeled as follows: $b = x + 0.1$ and $c = 2 \quad x$. We saw that the switch from ethanol to acetate was a progressive phenomenon with oxygen uptake. In anaerobiosis, small amounts of acetate were produced, resulting in an acetate coefficient of 0.15. This property was found among many strains of *Leuconostoc*.[13,18] As we expected when writing a balance on acetyl-P splitting on Figure 1, the values of acetate plus ethanol coefficient were constant (Fig. 6) and equal to 2.1. Instantaneous variations of this coefficient were similar to the previous variations of lactate and CO_2 coefficients.

Determination of Oxygen Coefficient

The relationship between the oxygen coefficient (x) and the acetate coefficient (b) determined here shows that the two coefficients are practically equal. This value reinforced our conclusion in respect to the assimilation of galactose via the Leloir pathway because it corresponded to the theoretical value which could be deduced from Figure 1. Indeed, for b mol acetate produced per mole of lactose, 2×2 mol of NADH, H^+ were needed to ferment glucose-6P to pentose 5P, and $2(2 \quad b)$ moles of NADH, H^+ were consumed to synthesize ethanol. A total consumption of $2b$ mol of NADH, H^+ per mole of lactose is encountered. Whichever of the NADH oxidase is activated, b mol oxygen are consumed per mole of lactose in this case, which effectively corresponds to the acetate production.

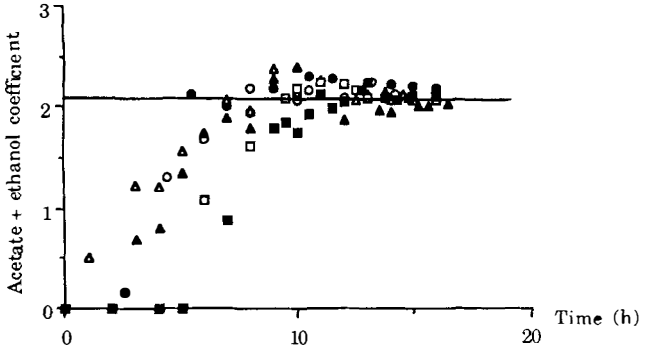

Figure 6. Acetate combined with ethanol coefficient for: (○) anaerobiosis; (●) air, 60 L/h; (●) air, 120 L/h; (▲) air, 240 L/h; (□) O_2, 36 L/h; (■) O_2, 120 L/h.

CONCLUSIONS

The oxygen effect was clearly demonstrated on each end-product concentration and general Equation (5) of lactose breakdown by a *L. mesenteroides* strain was deduced from experimental data as follows:

$$\text{lactose} + x\,O_2 \rightarrow 2\ \text{D-lactate} + (x + 0.1)\ \text{acetate} + (2 - x)\ \text{ethanol} + 2CO_2 \qquad (5)$$

This equation is very simple because it only depends on the oxygen coefficient. Coefficients of D-lactate, CO_2, and acetate plus ethanol together are equal and keep a constant value of 2 whichever the level of aeration: these end products are produced in equimolar quantities and their synthesis is independent of oxygen uptake. Yashima et al.[18] find the same conclusions for a fixed level of aeration when working on glucose breakdown by *Leuconostoc* species. As general Equation (5) shows, biochemical pathways of lactose catabolism are complex in the case of heterofermentative and facultative aerobic organisms. This work allows us to better understand the behavior of this strain in the presence of oxygen and can be useful when working on biomass production. Indeed, acetate production is closely linked to ATP production. Determining precisely the model which gives acetate production versus oxygen uptake seems to be an excellent way to predict maximal biomass production and the conditions of aeration which must be put into place to reach maximum biomass production.

Furthermore, Equation (5) provides some helpful information on galactose metabolism by an heterofermentative microorganism. Our results lead us to think that only the Leloir pathway functions with the strain studied. But it seems that the pathway activated can be very different with the strain used, even among heterofermentative microorganisms, according to the results of Premi et al.[15]

We thank Professor J. P. Riba for his helpful advice during the preparation of this manuscript.

NOMENCLATURE

a	stoichiometric coefficient of lactate = $MC_{lactate}/\Delta S$
b	stoichiometric coefficient of acetate = $MC_{acetate}/\Delta S$
c	stoichiometric coefficient of ethanol = $MC_{ethanol}/\Delta S$
d	stoichiometric coefficient of carbon dioxide = $CP/\Delta S$
x	stoichiometric coefficient of oxygen = $OU/\Delta S$
C^*	oxygen solubility (mol/L)
C	dissolved oxygen concentration (mol/L)
CP	carbon dioxide produced (mol/L)
CPR	carbon dioxide production rate (mol/L/h)
F_N	molar flow rate of inert gas (nitrogen) (mol/h)
F_{in}	molar flow rate of total inlet gas (mol/h)
F_{out}	molar flow rate of total outlet gas (mol/h)

kla	volumetric mass transfer coefficient (h^{-1})
M_i	molar concentration of an end product i in the medium (mol/L)
MC_i	$= M_i*V(t)/10$ (mol/L)
OU	specific oxygen uptake (mol/L)
OUR	oxygen uptake rate (mol/L/h)
OTR	oxygen transfer rate (mol/L/h)
$P_{O,out}$	partial pressure of oxygen in outlet gas (atm)
$P_{C,out}$	partial pressure of carbon dioxide in outlet gas (atm)
t	time (h)
V	medium volume (L)
$V(t)$	instantaneous medium volume, equal to $\beta t + \gamma$, with β and γ fixed (L)
ΔS	lactose uptake (mol/L)
C, H, O balances	initial = C, H, O (lactose) + O (oxygen) (mol/L); final = C, H, O (lactate) + C, H, O (acetate) + C, H, O (ethanol) + C, O (CO_2) (mol/L)

References

1. Bissett, D. L., Anderson, R. L. 1974. Lactose and D galactose metabolism in group N streptococci. Presence of enzymes for both the D-galactose 1-P and D-tagatose 6 P pathways. J. Bacteriol. **117**: 318–320.
2. Blackwood, A. C., Blakley, E. R. 1957. Metabolism of gluconate and 2-ketogluconate by *Leuconostoc mesenteroides*. Bacteriol. Proc. 121–122.
3. Chauvet, J., Brechot, P., Dubois, C., Dupuy, P., Dorange, J. L. 1982. Malic and citric acid stimulating growth of malolactic bacteria in wine. Sci. Aliment. **2**: 495 504.
4. Condon, S. 1987. Responses of lactic acid bacteria to oxygen. FEMS Microbiol. Rev. **46**: 269 280.
5. Cooney, C. L., Wang, H. Y., Wang, D. I. C. 1977. Computer-aided material balancing for prediction of fermentation parameters. Biotechnol. Bioeng. **19**: 55 67.
6. De Man, J. C., Rogosa, M., Sharpe, M. E. 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. **23**: 130–135.
7. DeMoss, R. D., Bard, R. C., Gunsalus, I. C. 1951. The mechanism of the heterolactic fermentation: A new route of ethanol formation. J. Bacteriol. **62**: 499 519.
8. DeMoss, R. D., Gunsalus, I. C., Bard, R. C. 1953. A glucose 6 phosphate dehydrogenase in *Leuconostoc mesenteroides*. J. Bacteriol. **66**: 10 16.
9. Gunsalus, I. C., Gibbs, M. 1952. The heterolactic fermentation. II. Position of C^{14} in the products of glucose dissimilation by *Leuconostoc mesenteroides*. J. Biol. Chem. **194**: 871 875.
10. Hurwitz, J. 1958. Pentose phosphate cleavage by *Leuconostoc mesenteroides*. Biochem. Biophys. Acta. **28**: 599–602.
11. Johnson, M. K., McCleskey, C. S. 1957. Studies on the aerobic carbohydrate metabolism of *Leuconostoc mesenteroides*. J. Bacteriol. **74**: 22 25.
12. Kandler, O. 1983. Carbohydrate metabolism in lactic acid bacteria. Antonie Van Leeuwenhoek **49**: 209 224.
13. Lucey, C. A. 1985. Active role of oxygen and NADH oxidase in growth and energy metabolism of *Leuconostoc*. M.S. thesis, University College, Cork, Ireland.
14. Plihon, F., Taillandier, P., Strehaiano, P. 1995. Oxygen effect on batch cultures of *Leuconostoc mesenteroides:* Relationship between oxygen uptake, growth and end products. Appl. Microbiol. Biotechnol. **43**: 117–122.
15. Premi, L., Sandine, W. E., Elliker, P. R. 1972. Lactose hydrolysing enzymes of *Lactobacillus* species. Appl. Microbiol. **24**: 51–57.
16. Romano, A., Brino, G., Peterkofsky, A., Reizer, J. 1987. Regulation of β galactoside transport and accumulation in heterofermentative lactic acid bacteria. J. Bacteriol. **169**: 5589 5596.
17. Thomas, T. D., Turner, K. W., Crow, V. L. 1980. Galactose fermentation by *Streptococcus lactis* and *Streptococcus cremoris:* Pathways, products, and regulation. J. Bacteriol. **144**: 672–682.
18. Yashima, S., Kawai, K., Okami, Y., Sasaki, Y. 1970. Effect of oxygen on glucose dissimilation by heterolactic bacteria. J. Gen. Appl. Microbiol. **16**: 543 545.